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July 15, 2016

RE: Availability of Eastern Equine Encephalitis and West Nile virus testing

Dear Vermont Veterinarian:

In recent years, various species of large animals in Vermont have been infected with Eastern equine encephalitis (EEE) virus or West Nile virus (WNV). There does not appear to be any part of the state that is more at risk than others for WNV, but both northwestern Vermont and portions of the Addison/Rutland County region are thought to be at elevated risk for EEE. Both diseases are usually severe in unvaccinated, susceptible animals. It is recommended that susceptible animals be vaccinated, and that people take precautions to prevent mosquito bites.

This summer, the Vermont Department of Health and the Vermont Agency of Agriculture, Food & Markets will again be offering testing of highly susceptible species, free of charge, from July 1<sup>st</sup> through November 15<sup>th</sup>. Specimens that meet the criteria for testing will be tested for both EEE virus and WNV.

**Criteria for testing through the Vermont Department of Health Laboratory:**

1. Onset of illness:
  - July 1<sup>st</sup> through October 31<sup>st</sup> (unless animal has compelling travel history)
2. Species:
  - Equids
  - Camelids (i.e. alpacas, llamas)
  - Ratites (i.e. emus)
3. One or more of the following symptoms:
  - Ataxia or stumbling and incoordination
  - Inability to stand
  - Acute paralysis or limb weakness
  - Sudden death with no other diagnosis
  - Severe hemorrhagic enteritis (emus)

We will ask you to provide the following information:

- Date of onset of illness
- Date of specimen collection
- Address where animal is stabled/housed
- Vaccination history
- Travel history
- Disease history
- List of neurologic symptoms



Note: All brain tissue samples from equids and camelids that test negative for rabies at the Vermont Department of Health Laboratory will automatically be sent for arboviral testing.

### Sample submission:

1. Animals that died or were euthanized:

- Brain tissue is the preferred sample, especially for non-equids and vaccinated animals. ***Before submitting samples for arbovirus testing, please call the Bradley Tompkins or Jillian Leikauskas at the Vermont Department of Health (802-863-7240) to confirm that the animal meets the testing criteria.*** Most of these animals will have to be tested for rabies first.
- Brain tissue samples collected for rabies testing can be used for arboviral testing as well. (Note: tissue samples should be refrigerated and kept cold during transport).
- Brain tissue samples should be sent to the Vermont Health Department Laboratory with the rabies sample submission form.
- If the sample is negative for rabies, the brain tissue sample will be sent to Wadsworth Lab in New York or the New Hampshire Department of Health Laboratory for EEE virus and WNV testing.

2. Animals that are alive:

- ***Before submitting samples for arbovirus testing, please call the Bradley Tompkins or Jillian Leikauskas at the Vermont Department of Health (802-863-7240) to confirm that the case meets the testing criteria.*** If the criteria for testing are met, a serum or CSF sample can be sent to the Vermont Department of Health Laboratory. Please use the lab submission form available at [http://healthvermont.gov/enviro/ph\\_lab/documents/Laboratory\\_Clinical\\_Test\\_Request\\_Form.pdf](http://healthvermont.gov/enviro/ph_lab/documents/Laboratory_Clinical_Test_Request_Form.pdf)
  - Check the box next to “Other” in the Serology Tests (Misc) section and write WNV/EEE on the line next to it.
  - Also write what kind of animal it is somewhere on the form (there is no designated place for this)
- Serum samples
  - Submit  $\geq 3$  ml of serum. IgM testing for both EEEV and WNV will be done on this sample<sup>1</sup>.
  - Serum should be collected within the first 14 days of illness.
  - Samples positive on the initial screening will be confirmed by PRNT.
  - Convalescent samples may be necessary and should be collected about 2 weeks later.
  - Serology can be difficult to interpret in vaccinated animals.
- CSF
  - Submit  $\geq 1$  ml of CSF. IgM testing for both viruses will be done on this sample.

### About EEE virus in animals

EEE virus is a mosquito-borne viral disease that causes a progressive neurologic condition in horses and other equids. Alpacas, llamas and emus are also known to be susceptible to illness. The mortality rate in horses is 75 to 90 percent. Clinical signs of EEEV in horses include fever,

depression, loss of appetite, weakness, ataxia, chewing movements, head pressing, circling, “sawhorse” stance, paddling, seizures, irritability, excitability, blindness, and abnormal sensitivity to light and sound. However, the illness in horses can also be peracute, and some die suddenly without showing obvious signs or symptoms.

The signs and symptoms are not unique to EEE. Other conditions to consider include West Nile virus encephalitis, tetanus, rabies, equine herpesvirus-1, equine protozoal myeloencephalitis, and western or Venezuelan equine encephalitis.


In emus, infection typically results in a rapid onset of clinical signs, often resulting in death. Common manifestations include disseminated intravascular coagulation, severe hemorrhagic enterocolitis, and blood-tinged vomitus. It is interesting to note that emus develop high levels of viremia. Unlike horses and humans, they may not be dead-end hosts and may act as a reservoir for the virus. There is evidence that the vaccines available for horses may protect emus and alpacas from infection with the EEE virus.<sup>1 2</sup>

Please remember that EEE in animals is a reportable disease in Vermont. If you have any questions, please call the State Veterinarian’s office at 802-828-2421 or the Health Department’s Infectious Disease Epidemiology program at 802-863-7240.

For more information on arboviruses and mosquitoes, please go to our websites:

<http://healthvermont.gov/prevent/arbovirus/index.aspx> - or -  
[http://agriculture.vermont.gov/plant\\_pest/mosquitoes\\_ticks/mosquitoes](http://agriculture.vermont.gov/plant_pest/mosquitoes_ticks/mosquitoes)

Thank you for your help.



Kristin Haas, DVM  
State Veterinarian  
Vermont Agency of Agriculture



Bradley Tompkins, MS, MPH  
Infectious Disease Epidemiologist  
Vermont Department of Health

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<sup>i</sup> IgM is species specific and can only be done on equids. For other species, a plaque-reduction neutralization titer will be done. This test is very specific, but it is a total antibody test that doesn’t distinguish IgM from IgG. Therefore, it cannot distinguish a vaccination titer from a disease response or acute disease from a past infection. It may be necessary to collect a convalescent sample to confirm the diagnosis.

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<sup>1</sup> Tengelsen, LA et al., “Response to and efficacy of vaccination against eastern equine encephalomyelitis virus in emus” JAVMA Vol. 218, No. 9, 2001

<sup>2</sup> Bedenice, D et al. “Humoral response to an equine encephalitis vaccine in healthy alpacas: JAVMA Vol. 234, No. 4, 2009